Art. XVIII.—*Osmotic Equilibration in the Living Body.*

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The primitive form of life from which all present existent forms of animal life are derived was, according to the Darwinian theory, a unicellular organism, analogous to the *Amoeba*, which, undoubtedly, lived in the ocean. From this form were evolved the simple coelenterates, and ultimately animals appeared which contained in their organisation a closed cavity which contained a fluid undoubtedly derived from the environment, the ocean. This closed cavity and its contained fluid were ultimately destined to become the circulatory system and the circulating fluid (blood) of the higher animals.

When these primitive animals left the sea and took to an estuarine life as a preliminary to living in the mud and, ultimately, on dry land, they took with them, contained in this body cavity, a fluid which had the same chemical composition as the ocean, their original home.

In the future evolution of this type of terrestrial animal, it is legitimate to postulate that the composition of this fluid remained about the same, and thus the animal, its cells attuned to a fluid of this composition, and by its terrestrial life removed from any chance of being exposed to any alterations in the composition or concentration of this circulating fluid, was given every opportunity of ascending in the scale of evolution.

We have here the first example of standardisation in the body, one of the principal stepping-stones to the proper and efficient evolution of the individual and race, a process well exemplified in the high degree of standardisation met with in the higher mammals, not only of the osmotic pressure of the

blood, but also the chemical composition, salt ratios, surface tension, and viscosity of the blood, as well as standardisation of body temperature, rate of heart beat, and rate of respiration ; the higher the animal the greater is the degree of standardisation of its various functions.

Those animals, however, which had remained in the sea, or the early descendants of the primitive terrestrial animal which had migrated back to this ocean which Macallum (6) places in precambrian times must, since those times, have had a constant fight against the increasing concentration of the ocean. On the other hand, those primitive creatures which had betaken themselves to the fresh water must have had a fight against the tendency to dilution of their body fluids.

With what mechanisms, then, is the animal organism endowed which enable it to oppose environmental influences affecting the osmotic pressure of its blood?

We know that one of the chief functions of the kidneys is to maintain the osmotic pressure of the blood constant, and, since in the mammal (for we know more of mammals and are better able to study them) there is a constant drain of water from the blood to supply the sweat and saliva, both very watery fluids (all other secretions being more or less isotonic with blood), the kidney is normally called upon to secrete a fluid whose osmotic pressure is generally very much above that of the blood. In this connection the results of Sommerfeld and Roder (13) are of interest. The osmotic pressure of the urine of a suckling fed on different milk diets was determined, and it was found that on a diet of full cow's milk the urine had a Δ of 0.736⁰C, and on its mother's milk the urine of the suckling gave a Δ of 0.746⁰C. But there is another means at the disposal of the organism, and that is by allowing entry into the alimentary canal of foods whose osmotic pressure is not above that of the blood, or in the case of sea animals (teleosts upwards), where this is not possible, either the non-absorption of the extra saline constituents of the sea-water, or if the absorption of these substances does take place, their elimination by the kidney. Bottazzi (1) has shown in the case of the marine teleosts that the latter process takes place, but whether marine mammals have adopted this practice, or whether they simply do not absorb

the excess of salt (and thus of necessity do work against osmotic pressure), has not been determined, but the fact that the Δ of the urine of seals recorded by Portier gives values of 0.73 to 3.5 seems to point to the fact that salts are absorbed and excreted by the kidney, the urine probably having a low Δ between meals and a high Δ during digestion, as suggested by Bottazzi (1). But in regard to land mammals in general and man in particular, my own observations on the osmotic pressure of liquid foods throw some light on the subject.

It is generally admitted that one of the functions of the stomach is the establishment of osmotic equilibrium between the fluid food swallowed and the blood (3). This it does by adding salts, etc., to hypotonic fluids, and by diluting the hypertonic. In the latter function the action of the stomach is aided by the salivary glands, which pour out a copious secretion of saliva. In one experiment cited in the appendix, 72 c.c. of saliva were poured out in 15 minutes during the process of chewing about 12 grms. of barley-sugar.

This function is no doubt protective, for the deleterious effects of exposing tissue cells to the action of hypertonic or hypotonic fluids are well known. The swelling up of, and interference with sensation in, and the function of the skin after prolonged immersion in water, and on the other hand the " roughness " produced when a piece of confectionery is retained for a few minutes between the teeth and cheek, are common examples.

Of the foodstuffs ordinarily admitted to the stomach, the great majority are in solid or gelatinous or colloidal form, and to such substances the considerations of osmotic pressure cannot apply. The actual fluid foods admitted to the stomach of man are milk, the ordinary beverages, fruit juices and beef teas, meat extracts and soups, while in the case of the lower animals in the natural state, the list is very much shortened, since the ingestion of prepared foods is solely confined to man and the domestic animals. In tea, coffee and cocoa there is usually a sugar addition, which varies with the personal taste, but the Δ given by the contents of a cup of tea, as prepared for an ordinary individual, was found to be about 0.46°C. The Δ of milk has been determined by many observers, and is the

same as the blood, 0.57-0.59. Of all the fluid foods admitted to the stomach of man, alcoholic beverages and fruit juices alone are hypertonic. In fact most of the fluid foods admitted to the stomach are hypotonic, thus containing a water excess which the organism readily avails itself of for purposes of " flushing out " the system, and which also allows of the addition of hydrochloric acid in the stomach. Moreover, it may be safely stated that in no case is a fluid admitted in which the hypertonicity is due to the mineral ingredients alone, except under protest. The sense of taste stands at the entrance to our alimentary canal, and tests the food not only qualitatively but also quantitatively from the standpoint of molecular concentration. To this function of the sense of taste we may give the name osmotactic, and it is undoubtedly to the possession by the sense of taste of this osmotactic character that the stomach mucosa is shielded from exposure to foods whose osmotic pressure is far above that of the environment to which the cells are normally accustomed. When for any reason a highly hypertonic fluid is admitted to the stomach, then vomiting (or sometimes diarrhoea) is the result. This is well seen after the ingestion of strong salt solutions, large quantities of sweetmeats or strong peptone solutions, or over-indulgence in alcoholic or strongly saccharine beverages. In the process of mastication and swallowing, the salivary glands are stimulated to the pouring out of a copious secretion, and the organism has developed here, too, another mechanism to aid in the dilution of the stomach contents—namely, the sense of thirst, the proper satisfaction of which, by the ingestion of a sufficient quantity of water, tends to the dilution and consequent lowering of the osmotic pressure of stomach contents. If these protective mechanisms fail in carrying out their object, then the organism responds by rejecting the contents of the stomach altogether by the reflex mechanism of vomiting.

Thus in man, at any rate, and undoubtedly in the case of the other mammals, the maintenance of a constant osmotic pressure of the blood by means of the kidneys and excretory organs is in a remarkable manner and to a considerable degree aided by the possession by these higher animals of the senses of taste and thirst. (Of man and mammals we can speak with

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certainty, although it is legitimate from common experience
to postulate that all vertebrates at least possess these senses
too.) The power to vomit is possessed by most animals.
Amongst ruminants and solipeds it is a rare occurrence, but,
after all, the nature of the food of these animals would un-
doubtedly exempt them from the necessity of ever rejecting
the contents of the stomach. But with man and the carnivores
particularly, the nature of their food, particularly the liability
to putrefaction and ptomaine formation, renders the existence
of the power to vomit a necessity, altogether excluding con-
siderations of the osmotic pressure of stomach contents, which
pertain chiefly to man, who, by an abuse of his intelligence,
perpetrates dietetic errors from which the lower animals are
protected by their instinct, and senses of taste and smell.

In connection with those hypertonic fluid foods which are
normally admitted to the stomach of man, the following obser-
vations of mine are of interest. A definite volume of beer was
taken which gave a Δ of 2.246, evaporated to dryness and
then calcined. The ash was then taken up in the same volume
of distilled water, and dissolved almost completely, leaving a
small residue of calcium carbonate, which probably existed in
the beer as the soluble calcium salt of some organic acid. This
watery extract gave a Δ of $0.026^{\circ}C.$; and similarly with the
juice of the orange—

        Δ of juice -   -   -   -   - 0.990
        Δ of aqueous extract of ash -   - 0.132

These experiments are analogous to what occurs in the body.
The saccharine and alcoholic constituents of the food are
rapidly absorbed and burnt off, or stored in the body as inert
substances (cf. glycogen), while the saline constituents are left
to play their part in the osmotic pressure phenomena in the
body. Thus we see that the kidney is constantly called upon
(in marine forms always and generally in land mammals as
well) to excrete inorganic salts. which tend to produce a rise
in the osmotic pressure of the blood, and thus the urine is
generally hypertonic. Prima facie, this means work done, and
hence in metabolism experiments this work done should be in-
cluded in the energy balance-sheet, for in some cases the amount
of energy thus expended may attain considerable dimensions
when converted into terms of heat value or mechanical work.

We thus see that in the evolution of the race there has been a steady growth of the tendency of the individual to become independent of its environment (and therefore not compelled to change in response to alterations in that environment) by means of maintaining through the co-ordinated and integrative action of all its organs, constancy in the action of its vital parts.

The removal of its vital organs from the disturbances of func- tion which must of necessity follow alterations in the physical and chemical properties of their environment, which in the higher animals are dependent on those of the circulating fluid, was the first great advance, and ensured for the organism the better and more efficient action of these organs, and afforded a distinct impetus to higher specialisation and greater independ- ence of external environmental change.   Thus, the beginning of the development of the firm resistant bony skeleton in place of the soft cartilaginous framework, and the formation of a high type of tissue from a simple one which this advance implies, most probably began to take place at a time in the history of the race when the cartilaginous fish was leaving the sea and taking to the mud of estuaries, where it most probably also developed the mechanism for maintaining the salt ratios and osmotic pressure of its blood a constant.

The sea water which is contained in the oceans of the present day gives a Δ of about 2.3°C. Bottazzi (1) gives a long series of determinations of the Δ of the body-fluids of many marine forms, and his results show that this Δ is approximately that of the sea-water environment in the case of coelenterates, echino- derms, crustaceans, gastropods, cephalopods amongst the inver- tebrates, and the elasmobranch fishes amongst the vertebrates.

Then there is a jump, and the blood of teleosts is found to give a Δ of about 1, and my own observations on the blood of the barracouta, a teleost inhabiting Australian waters, confirms these results.   (Δ of water of Hobson's Bay about 2.1.)   The next sea animals that we come to are the sea turtles, with a Δ of 0.7, and the next the sea mammals—seals, dolphins and whales.   The determinations of the Δ of the blood of these animals have been made by Portier (11), Rodier (12), and Jolyet (5), on the blood of animals which had been recently killed. Post-mortem decomposition may explain to a small degree the

large differences between the figure (0.59) for terrestrial mam-
mals, and the figures 0.64—0.71 for seals (Portier, 11), 0.8 for
dolphins (Jolyet, 12), and 0.74 for whales (Rodier, 5). Portier
(11) has also shown that the Δ of the blood of marine birds is
very little greater (0.63—0.69) than that of land birds (about
0.616, Bottazzi, 1).

With regard to fresh-water animals, the results are interest-
ing. Of invertebrates, I have determined the Δ of the body-
fluid of *Astacopsis bicarinatus*, which I find to be 0.616. Frede-
ricq (4) gives 0.8 for the body-fluid of *Astacus fluviatilis*, while
of vertebrates Bottazzi (1) gives : —

<blockquote>

Frog *(Ranaesculenta)*   -   -   -   0.465

Tortoise *(Emys europaea)*   -   -   0.44-0.48

</blockquote>

My own figures for the serum (from blood obtained by can-
nulisation of the carotid artery in the neck) of tortoises, *Emy-
dura macquariae* (Murray tortoise), at two different periods of
the year were 0.560 (June) and 0.550 (September).

The blood of various fresh-water teleosts, according to Ham-
burger and Dekhuyzen (quoted by Bottazzi 1), gives about 0.5,
while my own figure for the serum expressed from the blood
clot obtained from the vena cava of the Murray cod *(Oligorus
macquariensis)* is 0.650, which is perhaps a little high, due
to post-mortem decomposition.

Of terrestrial animals below true mammals I have two dif-
ferent determinations of the Δ of blood of *Echidna hystrix*
0.624, of an animal during hibernation, 0.600 of an animal
when awake, and *lizard* 0.639, and Bottazzi gives 0.616 as the
average Δ of the blood of land birds.

We are thus confronted with the following facts : —

(1)—Of marine animals, all forms below and including the
elasmobranch fishes possess a body fluid whose Δ is approxi-
mately that of the medium in which they live (Δ of sea-water
about 2.3ºC.), and Bottazzi (1) has shown that these animals
are poikilosmotic—i.e., the osmotic pressure of their body fluids
follows (within limits) that of the environment.

(2)—The blood of teleostean marine fishes gives a Δ of about
1ºC.

(3)—The blood of marine reptiles gives a Δ of about 0.7ºC.

(4)—The blood of marine birds has an osmotic pressure very
little higher than that of land birds (about 0.62—0.69ºC.)

(5)—Of marine mammals, seals (belonging to carnivora) give Δ very little above that of land mammals, and whales (cetacea) a somewhat larger Δ than seals and land mammals.

(6)—All terrestrial vertebrates examined, viz., reptiles (lizard), birds, echidna, mammals, possess a circulating fluid which gives a Δ of about 0.6ºC.

(7)—Of fresh-water animals the crustacean gives a Δ of 0.6 (0.8 according to Fredericq for *Astacus fluviatilis*). amphibia about 0.45, reptiles (tortoises) about 0.55ºC., and fishes (teleosts) 0.5—0.69ºC.

There are thus seen to be certain " jumps " in the figures given, which afford interesting food for reflection.

Firstly, with regard to marine animals, the question arises, why should the Δ of the blood of the teleosts differ in such a remarkable manner from that of the elasmobranchs? The acquisition of a bony skeleton by the teleost at once proclaims him a superior individual to the soft cartilaginous elasmo-branch. This fact combined with the indubitable fact that the environment of terrestrial life conduces in every way to a higher evolution, and the existence of the swimming bladder in these fishes, to my mind, lead to the conclusion that a carti-laginous fish passed through the stages of a mud fish, and ulti-mately lived in swamp, where it developed a bony skeleton, and meanwhile also developed the mechanism of maintain-ing the osmotic pressure of its blood constant. Some of these new individuals (bony fish) then wandered back to the sea, their original home, and the osmotic pressure of their blood gradually rose, but still has remained fairly constant near the original value. Others betook themselves to the fresh-water streams, and have maintained through the ages a blood whose osmotic pressure is approximately (possibly a little lower than) that of the teleost when it (as I presume) wandered back to the water. The sea turtle we can imagine to be a descendant of an individual which was evolved from this paludial teleost, and which wandered back to the sea at a later period than that in which the teleost returned to the sea.

The blood of marine birds and mammals is naturally ap-proximately that of the land birds and mammals, since these animals had taken to the sea in comparatively recent (geologi-

cal) times, and their organisation had been so constituted, that they have been able successfully to withstand the strain which their new environment has thrown on the lining membrane of the alimentary tract and on the cutaneous structures and kidneys in the maintenance of an osmotic pressure of their body fluid to which the protoplasm of their tissues had become attuned in the course of evolution.

In support of these conclusions may be brought the facts of palaeontology and zoology. The fishes of the cambrian and silurian ages were elasmobranch. These animals have a cartilaginous skeleton, and do not possess a swim bladder, although a few do possess a rudimentary diverticulum opening from the oesophagus. In the devonian period the dipnoi appear. The swim bladder is well developed, and the unused gills are degenerating. The animal is preparing for a terrestrial life, and we at once see the beginning of the development of the organs of phonation (the modern representatives, the mud fish of Queensland *(Ceratodus)* possessing a glottis arrangement in connection with the neck of the air sac), thus affording the animal the best means of communication with his fellows, and thus conducing towards a gregarious existence which is the basis of man's civilisation. In the strata of this era, too, are the fossil remains of the primitive amphibia, which most probably arose from these dipnoi. Then from the dipnoi evolved the holostei which existed in permian and triassic times, and were prominent in jurassic times. These no doubt passed on into the ganoids, which gave rise to the primitive teleostei which exist in the upper triassic and cretaceous ages and reached their full development in the upper eocene. By this time, no doubt, these fish had returned to the water, some to the sea, others to the fresh water streams, and in this return the swim bladder, now no longer needed for respiratory purposes, degenerated until in the higher teleosts the duct, which had been gradually shifting more dorsally (9), totally disappears, and the swim bladder remains in these animals a closed cavity, and is used by the animal for an altogether different purpose (that of a " sounding organ " and as a float). What determined the return of the evolving teleosts to the water cannot be stated, but the fact that fresh water fish belong exclu-

sively to the teleosts with the exception of one or two genera of stingrays in the rivers of tropical America (10) (which, after all, may be " accidental inclusions "), to my mind is strong argument in favour of this idea. Moreover, the development of the bony skeleton and the possession of a circulating fluid whose physical constants could be maintained irrespective of altera- tions in those of the environment enable these teleosts to migrate and pass from sea water into estuaries and up rivers, as is done by such a fish as the salmon in the breeding season, feats which would be practical impossibilities for the poikilosmotic elasmobranch.

Of course in considering this problem due consideration must be given to the method of " give and take " which is exemplified in all evolutionary processes. Thus could be explained any unaccountable differences in vascular develop- ment or higher development in parts of the nervous system in some of the lower animals, and as analogies may be taken the loss of the alimentary canal in intestinal worms and the degene- ration of the organs of smell in man when he assumed the erect posture.

The skeletons of whales appear in the tertiary periods (eocene and miocene), and since that time the whales have probably given in a little in the constant struggle against the concentra- tion of their body fluid by the surrounding medium. The seals probably appeared later than the whales, and possibly migrated to the sea later. This would account for the closer connection between the Δ of the blood of seals and the Δ of the blood of terrestrial mammals than holds in the case of the blood of whales. Marine birds are, probably by their aerial life and migratory habits (and hence ability to obtain fresh water from pools and rivers on land as suggested by Rodier, 12), removed from the necessity of " giving in " to the tendency of their food to render the body fluids hypertonic.

On the other hand, from reptile and bird to mammal, the osmotic pressure of the circulating fluid of the terrestrial animal is about the same (0.6). But why should the Δ of the body fluid of the fresh-water crustacean be much greater than that of amphibian and amphibious reptile (tortoise)? To me it appears that we have here evidence of a change of habit similar to what I imagine occurred to the fishes. A crustacean wandered

in comparatively recent times (Ortman (8), has shown that fresh-water crabs and crayfish did not appear till the upper tertiary) from the sea to the land and rivers, and finding food abundant and the environment favourable, remained there. In his new environment this poikilosmotic creature responded to the change in concentration of the medium in which he lived, and although the osmotic pressure of his body fluid has not yet reached that of the land animals derived from the primitive fish, it has, however, reached a very low figure indeed, when we take into account the fact that its tissues were originally attuned to an environment whose osmotic pressure was three or four times its present value. A similar line of reasoning will explain the lower osmotic pressure of the blood of the amphibia and to a small degree some amphibious reptiles.

The recently published work of Macallum (7) is of additional interest in this connection.

In contrasting the composition of the water of the oceans of the present day with the composition that the waters of the ocean at various geological epochs must have possessed, as determined from geological evidences, Macallum (6) had shown that the sodium content had rapidly increased, and the magnesium content had slowly increased, while, since rivers had formed, calcium and potassium had remained practically constant.

Macallum in his recently published paper (7) gives analyses of the blood serum and body fluid of various animals, and has also drawn up a table which gives the proportions of potassium, calcium, and magnesium to sodium, which is taken as 100. These tables I append.

TABLE A.

	Δ	Na	K	Ca	Mg	$\dfrac{{}^\circ Mg}{\Delta} \times 10$
Sea-water - - -		0.988	0.035	0.052	0.193	
Limulus (*Polyphemus*) -	2.04	0.8885	0.0499	0.0361	0.0996	488
Lobster (*Homarus ameri-canus*)	1.78	0.9034	0.0338	0.0439	0.0156	88
Dogfish (*Acanthias vulgaris*)	2.035	0.5919	0.0273	0.0161	0.0146	71
Cod (*Gadus callarius*) -	0.71	0.4161	0.0396	0.0163	0.0059	83
Pollock(*Pollachinus virens*)	0.825	0.4145	0.018	0.0129	0.00608	73
Mammals - - -	0.6	0.425	0.0226	0.011	0.004	67

*As calculated from Macallum's figures.

TABLE B.

	Na		K		Ca		Mg
Ocean - - - - -	100	-	3.66	-	3.84	-	11.99
Aurelia (*Flavidula medusa*) -	100	-	5.18	-	4.13	-	11.43
Limulus (*Polyphemus*) - -	100	-	5.62	-	4.06	-	11.20
Dogfish (*Acanthias vulgaris*) -	100	-	4.61	-	2.71	-	2.46
Lobster (*Homarus americanus*)	100	-	3.73	-	4.85	-	1.72
Pollock (*Pollachius virens*) -	100	-	4.33	-	3.10	-	1.46
Cod (*Gadus callarius*) - -	100	-	9.506	-	3.93	-	1.41
Dog - - - - -	100	-	6.86	-	2.52	-	0.81
Mammal - - - -	100	-	6.69	-	2.58	-	0.80

The latter table gives us some idea of the changes in salt ratios which have been taking place in the course of evolution. Ignoring the ratios of calcium and potassium, which do not show any marked variation, and confining our attention to the proportion of magnesium to sodium, we see that in the medusa (*Aurelia flavid.*), and the king crab (*Limulus*), the proportions are about the same as in sea-water. In the elasmobranch the proportion is 2.46 : 100, while the lobster (*Homarus americ.*) gives the proportion 1.72 : 100, teleosts (pollack and cod) give 1.46 and 1.41 : 100 respectively, while mammals give 0.81 : 100.

Magnesium is an element which exerts a markedly toxic action on protoplasm, and it is only natural to suppose that in the course of evolution not only would there be a fight against any alterations in the osmotic pressure and salt ratios of the environment, but that if the organism were compelled to yield, it would yield more in the case of the less toxic than in the case of the more toxic substances. Now the Δ of an animal's body-fluid is due to the presence in solution of organic bodies and salts (and their ions), and it may be assumed that the salts and their ions account for the greater part of this Δ.

On looking at the ratio Mg/Δ for the body fluid of various animals, we see that the dogfish, pollock and mammal give approximately the same ratio, the lobster a slightly higher figure, while the limulus gives a figure approximating that of sea-water.

Thus we see that, although the lobster and elasmobranch have not been highly enough evolved to resist the concentration of their body fluid, which the increasing concentration of their

environment would tend to produce, they have to a great degree been able to withstand the accumulation of the toxic magnesium in their body fluid.

The postulation of an extra-marine (fluviatile ?) existence for the ancestors of the lobster (as advanced supra) would account for this great difference between limulus and the lobster.

We thus see that the animal economy at the elasmobranch stage of its evolution is capable of resisting entry of toxic chemical substances into its organisation, but not of withstanding to any degree the physical forces of osmosis, so that, although the animal's economy can to some degree determine the salt ratios (and salt concentration too, as pointed out by Bottazzi, 2), in its body-fluid, it is unable to resist the concentration of this fluid by the osmotic pressure exerted by the environmental ocean.

A table showing the genealogical relationships of the fishes and higher vertebrates in accordance with the foregoing considerations and postulations is appended. (Appendix C.)

My thanks are due to Professor W. A. Osborne, under whose guidance this work was carried out.

APPENDIX A.

OSMOTIC PRESSURE OF LIQUID FOODS.

(Centigrade scale employed throughout.)

(Portions of the following results were published in the Biochemical Journ., 1909.)

Beverages.

Coffee (2 tablespoonfuls of sugar in ordinary breakfast cupful).—

$$\Delta$$
(1) - - 0.341°C.
(2) - - 0.343°C.

Tea infusion (2 teaspoonfuls (about 12 c.c.) of tea leaves in 200 c.c. boiling water, allowed to infuse 5 minutes).—

$$\triangle$$

(1) - - 0.052.
(2) - - 0.049
(3) - - 0.050

Tea (100 c.c. infusion, 50 c.c. water. 25 c.c. milk, 10 grms. sugar). Tasted " just nice."—

(1) - - 0.457
(2) - - 0.458
(3) - - 0.456

Tea (Second infusion, made with 200 c.c. more water added to leaves from infusion, and allowed to stand 35 minutes).—

(1) - - 0.026
(2) - - 0.025

Lemon juice (As used in Melbourne Hospital).—Strained juice of lemon (1 lemon) 33 c.c. in 250 c.c. distilled water.—

(1) - - 0.126
(2) - - 0.125
(3) - - 0.122

100 c.c. of diluted juice and 1 teaspoonful (5 grms.) cane sugar added. Tasted " just right."

(1) - - 0.487
(2) - - 0.485

" *Lime Juice and Soda.*"—(Marchant and Co., Melbourne.)—

(1) - - 1.268
(2) - - 1.265

Beer. (Carlton XXX. Draught Beer).—

(A) (1) - - 2.407
     (2) - - 2.409
(B) (1) - - 2.246
     (2) - - 2.250

30 c.c. of specimen (B) were taken and evaporated to dryness and calcined. Ash extracted with 30 c.c. distilled water. There was a small undissolved residue composed chiefly of calcium carbonate, which was probably derived from soluble salts of organic acids in the beer.

Ash Extract.—

(1) - - 0.026
(2) - - 0.026

Wine. (Cheap Australian claret). Cooled to —5°C, but ice would not separate out.

Wine.—Kept between 77°C and 80°C for 35 minutes, and then boiled briskly for about seven minutes to get rid of alcohol.

75 c.c. wine subjected to this treatment yielded 45 c.c.

(1)	-	-	3.240
(2)	-	-	3.238
(3)	-	-	3.241

Foodstuffs.

Treacle.—Diluted with water to 1 in 7, and this solution gave—

<p style="text-align:center">Δ
1.730</p>

Peptonised Milk.—Benger's peptonised milk, as used at the Melbourne Children's Hospital, Carlton, Melbourne, Victoria.

Benger's Milk " A."—(Milk 3, Water 1, peptonised 20 minutes. Boiled, sweetened with cane sugar about 1 oz. to 1 pint milk.) One drop gave pink biuret reaction.

| (1) | - | - | 0.652 |
| (2) | - | - | 0.656 |

Benger's Milk " B."—(Milk, 2, water 1, peptonised 20 minutes. Sweetened.)

(1)	-	-	0.630
(2)	-	-	0.628
(3)	-	-	0.626

Peptonised Milk.—(Milk 4, water 1, peptonised 20 minutes.)

| (1) | - | - | 0.548 |
| (2) | - | - | 0.546 |

Soups.—An ordinary soup which had been served up, but rejected as unpalatable on account of salt taste.

| (1) | - | - | 1.984 |

Soups.—

A vegetable soup was made of the following ingredients : —

| Carrot | - | - | - | 100 grms. |
| Parsnip | - | - | - | 110 ,, |

Turnip	-	-	-	55 grms.	
Spring onion	-	-	47	,,	
Celery	-	-	-	25	,,
Parsley	-	-	-	12	,,
Water (distilled)	-	-	1500 c.c.		

Brought to boil and kept simmering for 2¼ hours. Strained. Salted to different degrees.

Vegetable Soup Plain.—

(1)	-	-	0.374
(2)	-	-	0.373
(3)	-	-	0.372

Vegetable Soup Salted.—

A.	Soup + salt to 4 per cent. -	-	-	2.757	
B.	Soup + salt to 2 per cent. -	-	-	1.536	
C.	Soup + salt to ⅘ per cent. -	-	(1)	0.851	
				(2)	0.856
D.	Soup + salt to ¾ per cent. -	-	-	0.780	
E.	Soup + salt to ⅓ per cent. -	-	(1)	0.586	
				(2)	0.584
				(3)	0.582

The verdict of the taster was: The unsalted vegetable soup possessed a very flat and unsatisfactory taste. A, B, C and D distinctly too salty. A and B distinctly unpleasant taste. E was about right.

*Beef Tea.—*Made with about 6 c.c. meat extract (Fitzroy Brand, Queensland manufacture) in 1000 c.c. distilled water.

Beef Tea Plain.—

(1)	-	-	0.141
(2)	-	-	0.139
(3)	-	-	0.140

Beef Tea Salted.—

A. Beef tea + salt to 2.5 per cent.

(1)	-	-	1.626
(2)	-	-	1.626
(3)	-	-	1.625

B. Beef tea + salt to 1.25 per cent.

(1)	-	-	0.882
(2)	-	-	0.887
(3)	-	-	0.886

C.   Beef tea + salt to 0.625 per cent.

     (1) - - 0.546

     (2) - - 0.544

     (3) - - 0.543

D.   Beef tea + salt to 0.416 per cent.

     (1) - - 0.419

     (2) - - 0.416

Sample C was much the tastiest, just salted to taste ; A and B having too much salt, and sample D and the original not enough salt.

Beef tea made with about 6 c.c. meat extract in 1000 c.c. boiling distilled water.—

0.160

Beef Tea Salted.—Salt added till the flat and unsatisfying taste of the beef tea was abolished, but still no salty taste perceptible.

Taster A.   (1) - - 0.330

     (2) - - 0.331

Taster B.   (1) - - 0.329

     (2) - - 0.330

Beef Tea Over-Salted.—

1.922

Sugar Solutions.

Dextrose solution, 100 c.c. taken into mouth in sips of 25 c.c., each sip kept in mouth half minute, spat out ; in half minute another sip taken, kept in half minute, spat out ; and so on for four sips.

10 per cent. dextrose solution.

Δ

1.156

10 per cent. dextrose solution salivated.

     (1) - - 1.068

     (2) - - 1.066

5 per cent. dextrose solution.

0.566

5 per cent. dextrose solution salivated.

$$
\begin{array}{lll}
(1) & - & - & 0.536 \\
(2) & - & - & 0.532 \\
(3) & - & - & 0.534 \\
\end{array}
$$

Cane sugar solution, 20 grms. in 150 c.c. (13.3 per cent.), 100 c.c. treated in similar manner as dextrose solution above, 7 c.c. saliva were added by this process to the 100 c.c. sugar solution, and there was an after secretion for several minutes.

Cane sugar solution (13.3 per cent.).

0.868

Cane sugar solution salivated.—

$$
\begin{array}{lll}
(1) & - & - & 0.788 \\
(2) & - & - & 0.792 \\
\end{array}
$$

Fruit Juices.

Lemon.—Weight 140 grms. Peel and connective structure 90 grms. Yielded 40 c.c. strained juice.—

Δ

$$
\begin{array}{lll}
(1) & - & - & 0.937 \\
(2) & - & - & 0.940 \\
(3) & - & - & 0.939 \\
\end{array}
$$

Orange.— A. Orange 135 grms. Yielded 50 c.c. strained juice.

$$
\begin{array}{lll}
(1) & - & - & 1.100 \\
(2) & - & - & 1.101 \\
(3) & - & - & 1.100 \\
\end{array}
$$

B. 225 grms. peeled orange. Yielded 110 c.c. strained juice (pressed by hand).

$$
\begin{array}{lll}
(1) & - & - & 0.990 \\
(2) & - & - & 0.992 \\
\end{array}
$$

30 c.c. juice evaporated to dryness and calcined, and ash taken up in 30 c.c. distilled water. There was a small undissolved residue, composed chiefly of calcium carbonate.

Ash extract.—

$$
\begin{array}{lll}
(1) & - & - & 0.134 \\
(2) & - & - & 0.132 \\
\end{array}
$$

Pineapple juice.—From fresh Queensland pineapple.

$$
\begin{array}{lll}
(1) & - & - & 1.462 \\
(2) & - & - & 1.464 \\
(3) & - & - & 1.460 \\
\end{array}
$$

Cocoanut " milk."—(About 150 c.c. were yielded by the nut.)

(1)	-	-	0.521
(2)	-	-	0.518
(3)	-	-	0.518

Peaches.—300 grms. of peaches (5 stoneless peaches) yielded 180 c.c. strained juice.

(1)	-	-	1.090
(2)	-	-	1.088
(3)	-	-	1.088
(4)	-	-	1.090

Apricots and plums simply formed a pulp, but no juice was obtainable.

Cherry plums.—500 grms. cherry plums yielded 250 c.c. strained juice.

(1)	-	-	1.031
(2)	-	-	1.034
(3)	-	-	1.030

Tomato.—350 grms. tomato yielded 80 c.c. juice.

(1)	-	-	0.526
(2)	-	-	0.528
(3)	-	-	0.526

Apples.—300 grms. apple (ripe) yielded 30 c.c. juice.

(1)	-	-	1.196
(2)	-	-	1.192
(3)	-	-	1.195

Gooseberries.—300 grms. yielded plenty of pulp, but only 10 c.c. juice.

(1)	-	-	1.353
(2)	-	-	1.350
(3)	-	-	1.352

Cherries.—250 grms. yielded 105 c.c. juice.

(1)	-	-	2.246
(2)	-	-	2.244
(3)	-	-	2.243

Grapes.—250 grms. yielded 160 c.c. juice.

(1)	-	-	3.248
(2)	-	-	3.254

Saline Aperients.

Magnesium sulphate solution.—(15 grms. in 100 c.c.).

$$\Delta$$
$$1.136$$

Balanced saline aperient.—The stronger one as recommended by Professor W. A. Osborne in a paper in the Intercolonial Medical Journal of Australasia. July 20, 1909.

(1)	-	-	0.864
(2)	-	-	0.862
(3)	-	-	0.861

Saliva produced from sucking confectionery.—Barley-sugar stick (about 12 grms.) sucked for fifteen minutes led to production of 72 c.c. saliva.

(1)	-	-	1.008
(2)	-	-	1.006

Saliva from about 30 grms. boiled cane sugar, sweetmeat, 100 c.c.

(1)	-	-	1.488
(2)	-	-	1.484

The Beckmann freezing point method was employed throughout.

A mixture of ice and salt water was used to produce the requisite cold, but care was taken to prevent excessive supercooling.

In none of the recorded readings was the degree of supercooling more than about 1.5°C.

Crystallisation was started by inoculation with a fragment of frozen distilled water.

The stirring was carried out by a simple clock-work mechanism.

In the case of soups the " salting to taste " was carried out by a laboratory attendant, who was not aware of the purpose of the research.

5

APPENDIX B.

Comparative Cryoscopy of the Blood.

(Portion of the following results have been published in the
Biochemical Journal, 1911.)

(*The results of other authors are indicated by references.*)
Land Mammals—

		Δ		
Sheep (Defibrinated blood obtained from slaughter yards)	-	0.59	-	
Rabbit - - - -		0.59	-	
Echidna hystrix.—Hibernating (Septr.) (A) -	(1) 0.624		-	
	(2) 0.622		-	
Echidna hystrix.--Hibernating (May) (B) -	(1) 0.600		-	
	(2) 0.600		-	
Sea Mammals—				
Seals (*Phoca barbata*) -	0.64-0.66		-	Portier (11)
Whales (*Phocaena communis*) - - - -		0.74	-	Rodier (12)
Dolphin (*Tursiops tursio*)		0.83	-	Jolyet (5)
Land Birds—				
Domestic fowls - - -		0.616	-	Bottazzi (2)
Marine Birds—				
Larus glaucus - - -		0.69	-	Portier (11)
Uria troile - - -		0.66	-	Portier (11)
Eider - - - -		0.65	-	Portier (11)
Fulmarus glacialis - -	0.63-0.69		-	Portier (11)
Sea Reptiles—				
Sea Turtle - - -		0.7	-	Bottazzi (1)
Thalassochelys caretta -		0.61	-	Bottazzi (1)
Land Reptiles—				
Tortoises. *Emys. europea* -		0.474	-	Fredericq (4)
Emydura macquaria A	(1) 0.559		-	
	(2) 0.561		-	
	(3) 0.560		-	

	(B)	(1) 0.550	-		
		(2) 0.545	-		
		(3) 0.550	-		
Lizard. Mixed blood of		(1) 0.639	-		
Egerna cunninghami					
and a species of *Tiliqua*		(2) 0.637	-		

Amphibia—

Rana esculenta	-	-	0.465	-	Bottazzi (1)

Fish—Elasmobranchs (sea-water)—

Torpedo marmorata -		-	2.26	-	Bottazzi (1)
Mustelus vulgaris	-	-	2.36	-	Bottazzi (1)

Teleosts (sea water)—

Charax puntazzo	-	-	1.04	-	Bottazzi (1)
Cerna gigas	-	-	1.035	-	Bottazzi (1)
Crenilabrus pavo	-	-	0.74-0.76	-	Bottazzi (1)
Box salpa	-	-	0.82-0.88	-	Bottazzi (1)
Barracouta	-	A	(1) 0.979	-	
			(2) 0.978	-	
(*Thyrsites atua*)	-	B	(1) 0.976	-	
			(2) 0.980	-	

Teleosts (fresh water)—

Anguilla vulgaris	-	-	0.58-0.69	-	Bottazzi (1)
Barbus fluviatilis	-	-0.475-0.558		-	Bottazzi (1)
Perca fluviatilis	-	-	0.512	-	Bottazzi (1)
Murray Cod (*Oligorus mac-*					
quariensis) -	-	-	0.642	-	
			0.660	-	
			0.650	-	

Cephalopods—

Octopus macropus	-	-	2.24	-	Bottazzi (1)

Crustacean (Marine) —

Maja squinado	-	-	2.36	-	Bottazzi (1)
Homarus vulgaris	-	-	2.29	-	Bottazzi (1)

Crustacean (Fresh water)—

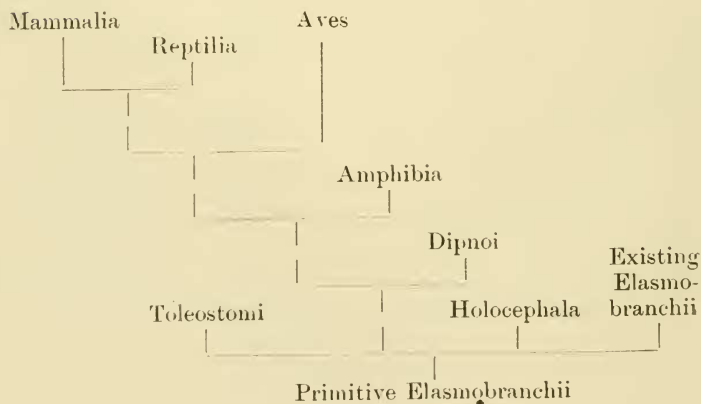|					
Astacus fluviatilis	-	-	0.80	-	Bottazzi (1)
Astacopsis bicarinatus	-	(1) 0.616	-		
		(2) 0.611	-		
		(3) 0.618	-		

J. L. Jona:

APPENDIX C.

From Parker and Haswell (6) and the Cambridge Natural
History, Vol. VII., ed. by S. F. Harmer and A. E. Shipley,
and Ortmann (8).

Eozoic	Archean				
Palaeozoic Primary — Newer Palaeo	Cambrian	Elasmobranch			
	Ordovician				
	Silurian	Elasmobranch			
Palaeozoic Primary — Older Palaeo	Devonian	Holocepheli	Chondrostei	Dipnoi	Amphibia
	Carboniferous			Dipnoi	
	Permian			Holostei	Reptiles
Mesozoic Secondary	Triassic	Mammals		Holstei	
	Upper Triassic				Teleost
	Jurassic	Mammals	Birds	Holstei	
	Cretaceous		Crab		Teleost
	Upper Cretaceous		Fresh-water Crab		
Cainozoic Tertiary	Eocene	Whales			Teleost
	Oligocene				
	Miocene	Whales			
	Pliocene				
	Postpliocene				

GENEALOGICAL TABLE

(according to Parker and Haswell).

Mammalia   Aves

Reptilia

Amphibia

Dipnoi   Existing Elasmobranchii

Toleostomi   Holocephala

Primitive Elasmobranchii

TABLE

modified in accordance with considerations contained in the
foregoing paper.

```
Mammalia (Triassic)   Aves (Jurassic)
      |_____|
              |                    Teleostei (Triassic)
        Reptilia (Permian)                 |
              |                             |
        Amphibia (Devonian)      Holstei (Permian)
              |_____|
                      |
              Dipnoi (Devonian)
                      |
              Chondrostei (Devonian)
                      |
              Holocephali (Devonian)  Existing Elasmobranchii
                  |_____|
                              |
                  Primitive Elasmobranchii
                  (Cambrian and Silurian)
```
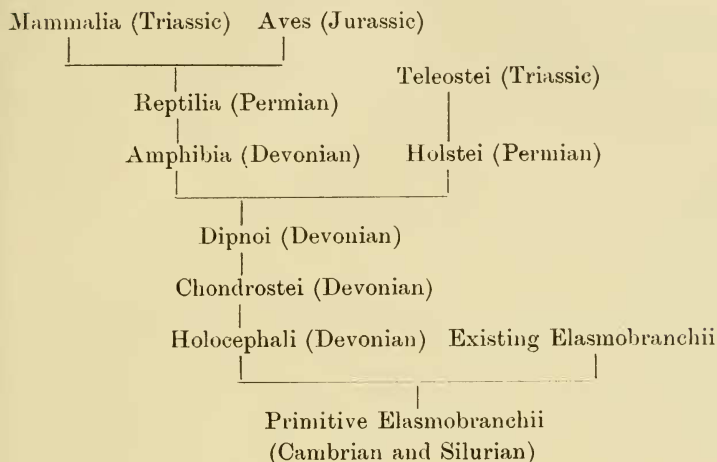
REFERENCES.

1. Bottazzi.—Ergebnisse d. Physiol., 1908, S. 246, et. seq.

2. Bottazzi.—Ergebnisse d. Physiol., 1908, S. 267.

3. Bickel.—Handbuch d. Biochem. (hgb. von Oppenheimer), Bd. III., 1te Hälfte, S. 86.

4. Fredericq.—Arch. Biol. (XX.). 1904, p. 709.

5. Jolyet.—Comptes rend. Soc. d. Biol., 1902, 54, p. 293. (Quoted by Portier.)

6. Macallum.—The Paleochemistry of the Ocean. Trans. Canad. Instit., Vol. VII. (1902), p. 532.

7. Macallum.—Proc. Roy. Soc., vol. 82 B, p. 602.

8. Ortmann.—Geographical Distribution of Decapods, Proc. Amer. Philosoph, Soc., Vol. XII., No. 171, p. 387.

9. Parker and Haswell.—Text Book of Zoology, 1897 ; Vol. II., p. 516.

10.   Parker and Haswell.—Ibid, p. 600.

11.   Portier, P.—Pression Osmotique des Liquides des Oiseaux et Mammiferes Marins, J. de Phys. et de Path. Gen., 1902, p. 202.

12.   Rodier.—Bull de la Soc. Scientifique d'Arcachon, 1900. (Quoted by Portier.)

13.   Sommerfeld and Roder.— Berl. Klin. Woch, 519, 21, p. 544 (1902).